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Textile Research Journal 2002; 72; 579
DOI: 10.1177/004051750207200704

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ABSTRACT

The use of atomization or an aerosol formation is investigated as a vehicle for enzyme treatment of dew-retted flax fibers. A cellulase/endoglucanase from *Trichoderma reesei* is applied by atomization to dew-retted fibers at concentrations of 0, 50, and 100 U. Enzyme activity post-atomization is verified by GLC analyses of sugars hydrolyzed from the treated fibers. Cellulase treatment results in the release of glucose and galactose moieties in a dose response manner. Fiber strength determination also demonstrates a dose response effect in relation to treatment with 50 and 100 U of enzyme, yielding strength decreases of 17 and 56%, respectively. Visualization of treated flax fibers by polarized light microscopy reveal the presence of structural aberrations previously identified as nodes, and treatment with the atomized enzyme coincides with fiber disruption at these regions. Mid-IR attenuated total reflectance spectra of fiber mats show a reduction in bound water and loss of acetylated components at the fiber surface resulting from enzyme treatment. Collectively, these results demonstrate the effectiveness of atomization as a means of applying enzymes for fiber modification, and the results also have implications for the flax fiber structure.

Using pure enzymes or mixtures of enzymes to isolate bast fibers from flax stems is currently being investigated as a means to expand flax production and to improve flax fiber uniformity and efficiency of flax processing. Currently, dew retting is the predominant method of retting flax, particularly in Northern and Eastern Europe where the climatic conditions are conducive for producing high quality dew-retted fibers [11, 13, 26, 27, 30]. Enzyme retting, however, is independent of weather and would circumvent the ecological and, therefore, the geographical constraints required for dew retting. Enzyme retting could result in growth of the flax industry and expand production to the U.S. However, a commercially viable enzyme retting technique is not currently available, and while these technologies are being developed, dew-retted flax from Europe remains the primary source of fibers for textile applications.

Dew-retted fibers result from the action of indigenous soil fungi, which colonize the harvested plant stems and degrade the pectic and hemicellulosic components of the stem through the action of extracellular polygalacturonases, xylanases, and arabinases [5, 6]. The degradation of these interstitial matrices results in the disassociation of fiber cells from their surrounding soft tissues, and subsequently allows for mechanical isolation of the cellulosic flax fibers by scutching and hackling [7, 12, 28, 29, 32]. Dew-retted flax fibers exhibit significant variations and are often coarse and of low quality [31]. Factors that may contribute to this lack of consistency include sporadic environmental conditions, inconsistent colonization by indigenous soil fungi, activity by cellulolytic organisms, and lack of an accurate means to determine the end point of retting [2, 19, 28, 29, 32].

The lack of consistency and quality in dew-retted fibers can affect downstream textile applications and the quality of the resulting product. This phenomenon is particularly apparent when dry spinning short stapled or "cottonized" flax fibers in blends with cotton. During this process, residual shive, cuticle, and surface waxes may limit interaction of fibers and thereby decrease spinning efficiency/production rate and reduce the strength of the resulting yarns [10]. Furthermore, the presence of these components can affect downstream processes such as bleaching and/or dyeing [18]. Research by Sharma indicated that treatment of flax roves with crude pectinolytic enzyme preparations to remove fiber contaminants enhanced the quality of resulting yarns [25]. In this work, enzymatic treatment consisted of submerging the roves

in the enzyme solution prior to wet spinning of the flax. Enzyme treatment by this method is not appropriate, however, for the dry spinning process currently used to process short staple flax fiber and flax/cotton blends and would require sufficient alteration of the flax processing line and equipment.

Atomization is currently used in therapeutic applications that involve a uniform dispersion of extremely small amounts of protein- or peptide-containing solutions (*i.e.*, aerosol formation) [23]. The process, which relies on the shearing action produced by passing air or gas over a solution and then forcing it through a small orifice, results in droplet formation where droplet size within the aerosol is inversely related to air or gas pressure [22]. Enzyme application with this process may also be applicable for enzymatic treatment of dew-retted flax fibers, since the atomized treatment would involve only a limited amount of moisture. However, to our knowledge, using this system for this kind of treatment has not been previously tested. Therefore, our objective in this study is to test atomization as a vehicle for the enzymatic treatment of dew-retted flax fibers for modifying fiber properties.

Materials and Methods

Dew-retted flax for cellulase treatment was obtained from an unknown cultivar grown, harvested, and dew retted in the Czech Republic and processed through the Unified Line of CML (Humpolec, Czech Republic). For further refinement, the dew-retted fibers were processed on Temafa's Lin Line (Bergisch Gladbach, Germany), producing shortened, refined fibers. For enzyme treatment, 5-g aliquots of processed dew retted fibers were evenly pulled into flat sheets and affixed to a crude frame (14 × 25.5 cm) with clips. Nonretted flax fibers were the Ariane variety and were isolated by hand in 1-cm sections from the middle section of the stems.

Cellulase/endoglucanase (EC 3.2.1.4) from *Trichoderma reesei* was obtained from Sigma Chemical Co (St. Louis, MO). All cellulase formulations were freshly prepared in 5 ml aliquots of 50 mM sodium acetate buffer (pH 5.0) and contained 0, 50, or 100 U of cellulase activity where 1 unit (U) of activity is defined as the amount of enzyme required to liberate 1.0 μmole of glucose in 1 hour from cellulose at pH 5.0 and 37°C. For the atomization treatment, each solution (5 ml) was uniformly applied to the 5 g samples of dew-retted flax fibers at room temperature by a Badger Crescendo™ air brush (model 175-7™, Badger Air-Brush Co., Franklin Park, IL). Pressure was maintained at 30 psi throughout the delivery of each solution and was provided by a compressed air tank. Following atomized buffer or cellulase (50 or 100 U) treatment, samples were individually sealed in ZipLoc® brand freezer bags (S.C. Johnson & Son, Inc, Racine, WI) to maintain humidity levels. Treated samples were then incubated at 37°C for 20 hours. Following incubation, 4 g of each sample were placed on aluminum trays and heated to 65°C for 30 minutes to inhibit further cellulase activity. The remaining 1 g samples were individually submerged and mixed in 10 mL of sodium acetate buffer (pH 5.0). From these solutions, 5 mL samples were immediately freeze-dried and the particulate used for later analysis. For immersion treatments, 1-cm sections of fibers hand-pulled from the middle of five dried, mature, nonretted Ariane flax stems were immersed in 10 mL of 50 mM sodium acetate buffer (pH 5.0) containing 0 or 50 U of cellulase activity ($n = 3$) and incubated at 37°C for 24, 48, or 72 hours.

Cellulase activity on the dew-retted fibers was monitored from the sugar composition of the freeze-dried particulates derived from the post-reaction buffer washes (5 ml) by gas liquid chromatography (GLC) as per Hoebler *et al.* [17]. Further effects of the atomized cellulase on the dew-retted flax fibers were determined by measuring the fiber properties of the resulting samples. Fiber strength and elongation were determined by Stelometer using the flat bundle method [1, 3]. Fiber fineness was analyzed by the airflow (micronaire) method modified to use a 5 g substrate based on flax calibration standards [1, 4].

Structural differences of untreated, buffer treated, and cellulase treated fibers were analyzed by polarized light microscopy and mid-IR spectroscopy. For mid-IR spectroscopy, infrared spectra were obtained with a DuraScope (SensIR Technologies, Danbury, CT) attenuated total reflectance (ATR) device in a Magna 850 (Thermo Nicolet, Madison, WI) Fourier-transform infrared (FTIR) bench. The bench was equipped with a KBr beamsplitter and a DTGS detector. Mats of flax fibers were placed on the surface of the diamond ATR crystal. Pressure was applied to the sample with a transparent rod until "wetting" of the sample on the crystal, as observed on the video monitor of the device, was assured (load of 5). Interferograms were collected at a resolution of 8 cm^{-1} over 4384 scan points at a scan velocity of 0.3165 cm/s to cover the range of 4000-650 cm^{-1}. The bench aperture was set at 150 (~15 mm) and the gain at 1.0. For each spectrum, 128 scans were co-added. After apodization with a Happ-Genzel function, the data were Fourier transformed and displayed in the absorbance mode against the background of the clean diamond crystal with no sample and no pressure applied. Three spectra from different locations on the fiber mat were obtained and averaged for the final spectrum file with no ATR correction.

Results and Discussion

Dew-retted flax fibers generally consist of 60–80% cellulose and so are susceptible to cellulolytic enzymes [11, 12]. Previously, cellulase treatments were used to free tightly bound proteins from the microfibrils of dew-retted flax. In this earlier study, treatment was by immersion of the flax fibers in a cellulolytic solution, and protein extraction was induced by the degradation of the cellulolytic fibers [11]. Therefore in this study, we have selected a cellulase/endoglucanase (E.C. 3.2.1.4) from *Trichoderma reesei* as the model enzyme to test the application of atomization or aerosol formation toward enzymatic treatment of dew-retted fibers.

Initially, dew-retted flax fibers were treated by atomization with 0, 50, or 100 U of cellulase, and enzyme activity was monitored for the resulting hydrolyzed sugars by GLC. Because treatment with the atomized cellulase occurred in a water-limited ("dry") environment, no supernatant was available for analysis. Therefore, we subjected the fibers to a post-treatment wash with 50 mM sodium acetate buffer, and determined the sugar content of the resulting washes. Results indicated that treatment with 0, 50, and 100 U of atomized cellulase yielded 0.017, 0.049, and 0.073 g of glucose per 100 g fibers, and 0.0048, 0.006, and 0.0085 g of galactose per 100 g fibers, respectively. No change in mannose, rhamnose, arabinose, or xylose content occurred due to treatment. The apparent dose-response effect of cellulase as estimated by glucose content in the washes was indicative of cellulolytic activity and was regarded as the initial proof of enzyme activity following atomization. The galactose content of the washes was also directly related to the level of cellulase treatment. Cellulolytic enzymes should not directly catalyze the release of galactose or galactans. However, previous research has shown that galactose is the main neutral sugar in polysaccharides linked to cellulose, and cellulose degradation likely resulted in galactose release [9, 14].

Mid-IR analysis of dew-retted fibers treated with atomized-cellulase at 0, 50, and 100 U indicated subtle changes at the fiber surfaces that could be related to enzyme treatment. After enzyme treatment, lower absorbances occurred in two regions (Figure 1). Absorbance near 1635 cm^{-1}, which is indicative of bound water, gave a similarly reduced signal for both enzyme treatments compared to the untreated and buffer control dew-retted fibers [8]. The reduction in bound water could indicate that amorphous or disordered components at the fiber surface were easily removed by low levels of enzyme activity, negating a dose response effect. Absorbance near 1250 cm^{-1}, which is indicative of C-O stretching of acetyl groups (likely present in noncellulosic carbohydrates, *e.g.*, an acetylated galactan), did show a dose response for the two enzyme levels [8]. This could indicate that noncellulosic materials, more than could be considered amorphous or disordered material, were further removed by an increase in enzymatic action. Either further removal of surface cellulose released closely associated materials or, alternatively, there are contaminating activities in the cellulolytic preparation that lead to this observation. The results are presented relative to the carbohydrate C-O stretch band near 1030 cm^{-1} (Figure 1) [8]. Although these changes were apparent in noncellulosic components, treatment with atomized cellulase at both 50 and 100 U did not appear to have a significant effect on the overall cellulosic chemistry of the dew-retted flax fibers based on spectra data. This apparent lack of effect could be due to extensive enzyme dispersion or perhaps to the presence of noncellulosic constituents, including hemicellulose, pectin, lignin, fat, wax, and protein associated with the fibers [12,

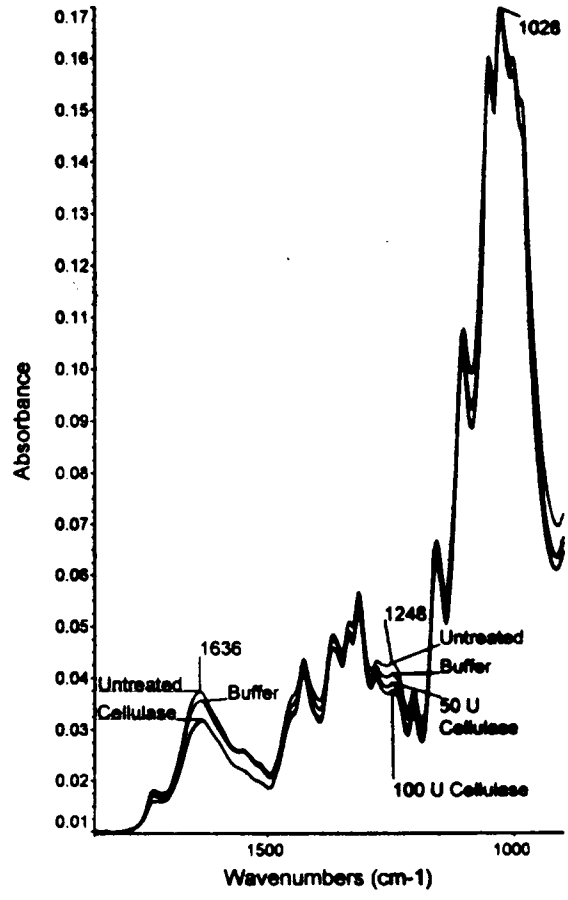

FIGURE 1. Mid-infrared attenuated total reflectance spectra in absorbance mode of untreated, buffer, 50 U cellulase, and 100 U cellulase treated dew-retted flax fibers. Bands for bound water (1636 cm^{-1}), acetyl C-O stretch (1248 cm^{-1}), and carbohydrate C-O stretch (1028 cm^{-1}) are indicated for the untreated sample.

20, 24, 25, 31]. Because these components are tightly associated with the flax fibers, their presence might impede enzyme/substrate interaction and thereby activity.

Polarized microscopy of treated and untreated samples revealed the presence of flax fibers as primarily dissociated ultimate fibers with the intermittent occurrence of small intact bundles (Figures 2a–d). The micrographs also illustrate the presence of structural aberrations in the fibers and fiber bundles, which have been previously identified as nodes [16, 21, 24], kink bands, or shear bands [33]. These structural defects are thought to result from microbuckling, which can occur along the fiber in the presence of an axial compression either during growth [21] or during processing [33]. A comparison of the untreated (Figure 2a) and buffer treated (Figure 2b) dew-retted flax fibers indicated no apparent differences due to treatment. In both samples, fibers remained intact and nodes were depicted as seams perpendicular to the length of the fibers. Treatment with 50 (Figure 2c) or 100 U (Figure 2d) of atomized cellulase had no perceivable effect on fiber shafts. However, both treatments paralleled disruption of the fibers at regions consistent with nodes. While the fibers treated with 50 U atomized cellulase exhibited slight separation at the node region (Figure 2c), fibers treated with 100 U atomized cellulase were often completely ruptured (Figure 2d). For both treatments, this action was erratic because not all nodes were visibly affected by the cellulase treatments.

To further investigate the deteriorating effect of cellulase against flax fiber strength, sections of hand-pulled nonretted flax fibers (Ariane variety) were incubated by immersion in a solution containing 50 U of cellulase activity. After 24 hours, evidence of hydrolysis was apparent (Figure 3) and continued incubation (48 and 72 hours) resulted in total fiber degradation (data not shown). Fiber rupture appeared to be initiated at the nodes and then extended along the shaft until the fiber was completely hydrolyzed.

We compared cross sections of untreated (Figure 4a) and atomized cellulase (100 U) treated (Figure 4b) fibers/fiber bundles by polarizing light microscopy in order to evaluate any internal changes to the fibers due to enzyme treatment. Deformations in fibers, showing lack of internal cell wall material and disruption of cell walls, occurred in both sample preparations, making any judgement as to possible effects by cellulase difficult to visu-

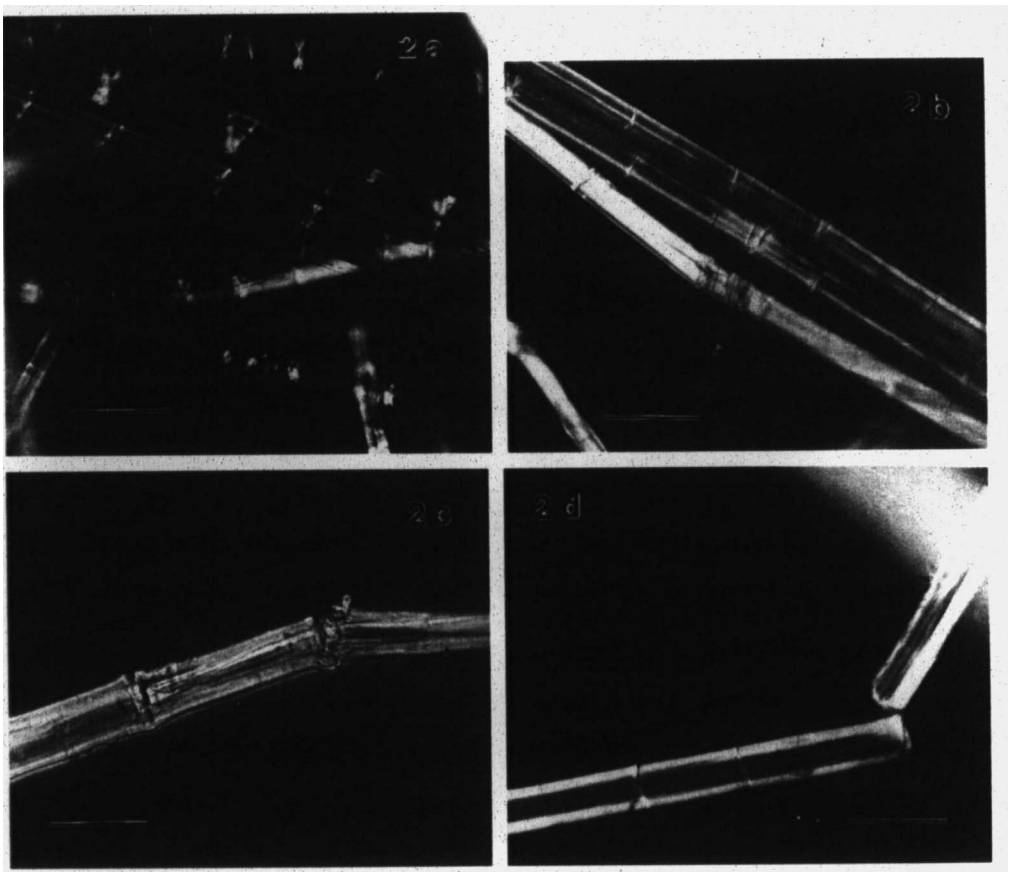

FIGURE 2. Polarized light microscopy of untreated (a), buffer treated (b), 50 U cellulase treated (c), and 100 U cellulase treated (d) dew-retted flax fibers. Bar = 100 μm, Figure a–b, bar = 50 μm, Figures c–d.

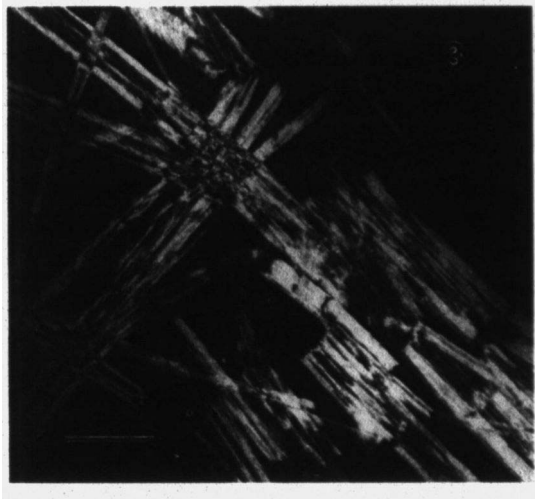

FIGURE 3. Polarized light microscopy of hand-pulled Ariane flax fibers treated with 50 U cellulase (24 hours). Bar = 200 μm.

alize. The figures do, however, reveal the internal structure of the flax fibers and show the substantial cellulosic structure present in both sets of fibers.

We determined strength and elongation properties for the treated fibers, and our results demonstrate a dose-response treatment effect (Table I). Treatment with 50 or 100 U of atomized cellulase yielded strength reductions of 17 and 56%, respectively, compared to the buffer control, and the 100 U cellulase treatment significantly ($p \leq 0.05$) reduced elongation (%) of the treated fibers. Interestingly, when compared to an untreated control, buffer (0 U) treatment reduced the strength of the dew-retted fibers by 22%. Because dew-retted flax fibers are known to contain large quantities of microbiota, it is very likely that buffer treatment and the subsequent incubation may have provided a proper environment for growth of the microbiota, which could have weakened the fibers through metabolic activities [15].

In summary, the physical and chemical tests presented here indicate that atomization is an effective enzyme delivery mechanism for treating dew-retted flax fibers. The GLC and strength analyses offer proof of residual activity post-atomization of the cellulolytic enzyme. We make no claims, however, about the effect of the process on the enzyme itself since we made no effort to directly measure enzyme activity pre- and post-atomization. Previous work has demonstrated adverse effects resulting from forming aerosols from enzyme solutions [23]. In addition, the lack of an aqueous environment and thereby water activity could limit enzyme efficiency, if not by rate then by extent, since the atomized enzymes would largely be immobilized on the fibers and unable to contact a new substrate. Increased humidity may serve as a means to further increase enzyme activity, and future work needs to identify the appropriate environmental conditions.

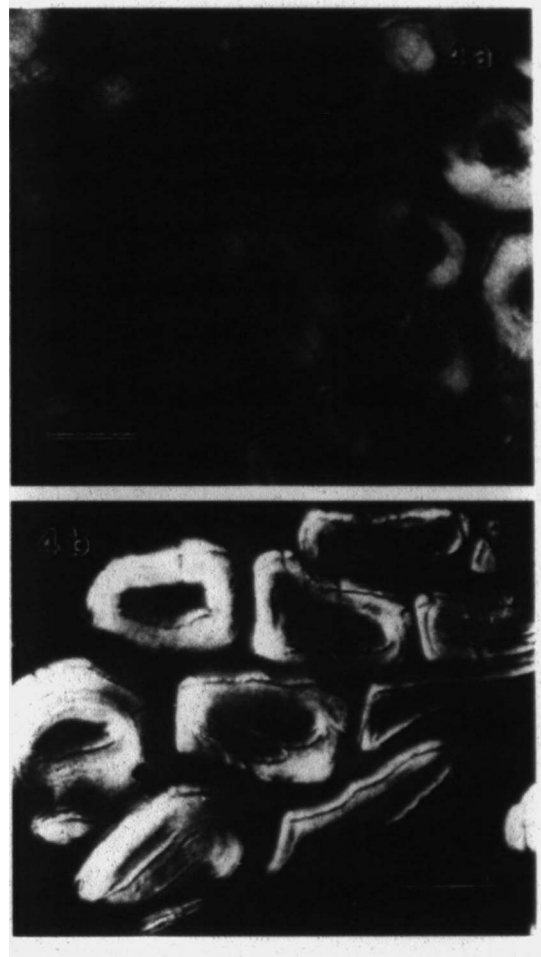

FIGURE 4. Polarized light microscopy of cross sections of untreated (a) and 100 U cellulase treated (b) dew-retted flax fibers. Bar = 20 μm.

TABLE I. Effect of atomized cellulase on dew-retted flax fibers. Average and standard deviation of three replicates, each replicate an average of six tests by Stelometer. Superscripts a, b, c, d indicate values within columns with different letters differ at $P \leq 0.05$.

Treatment	Strength, g/tex	Elongation, %
No treatment	29.2 ± 1.0[a]	1.1 ± 0.2[a]
Buffer (0 U) treatment	22.8 ± 1.4[b]	0.8 ± 0.3[a]
50 U Cellulase	18.8 ± 1.6[c]	0.8 ± 0.3[a]
100 U Cellulase	9.9 ± 2.0[d]	0.08 ± 0.2[b]

The use of the cellulolytic enzyme in this study also gives rise to interesting questions about the flax fiber structure itself. While strength and GLC data indicated that the enzyme had altered the physical structure of the fiber, polarized light microscopy implicated the nodes as the site of enzyme activity and not the fiber shafts. According to mid-IR analysis, the fiber shafts have associated noncellulosic materials, and these may act to

shield the fiber shaft from cellulolytic attack. However, the nodes, which appear as cracks in the fiber-associated materials, may provide access for the enzyme. Alternatively, the cellulose structure itself within the fiber shaft and at the node region may be implicated. However, further work is necessary to draw firm conclusions.

Conclusions

Atomization can be used to deliver effective doses of enzymatic solutions to dew-retted flax fibers to modify their properties. Furthermore, the low level of applied solution associated with the system should minimize cost, drying time, and fiber entanglement, all of which impede dry spinning of short staple flax fibers and flax/cotton blends.

ACKNOWLEDGMENTS

We would like to acknowledge the technical support provided by Luanne Rigsby and Ashley Heyward.

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Manuscript received October 25, 2001; accepted December 17, 2001.

Analysis of Energy Dissipation in Twisted Fiber Bundles Under Cyclic Tensile Loading

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ABSTRACT

This study of energy loss due to friction in dynamic loading conditions provides a new approach to characterizing fiber-on-fiber friction. Based on the theory of dynamic mechanical behavior of polymeric materials, the systematic experimental study uses cotton and polyester rovings to examine the effect of twist level, cyclic loading magnitude and frequencies, and gauge length on frictional energy loss. The test results are analyzed in comparison with Murayama's dynamic mechanical model. The model does not fit the experimental data, and a modification is proposed for characterizing the frictional energy loss of twisted staple fiber structures.

As one of the most important fiber characteristics, fiber-on-fiber friction affects the behavior of fibers in textile manufacturing processes and the performance of the final products [1, 6, 8, 11, 12]. Due to its paramount importance, much attention has been paid to fiber-on-fiber friction [2–5, 7, 9]. Almost all the approaches to date have measured the frictional force in either a tensile or a compressive test on a fiber bundle or the like. The frictional properties of fibers are then characterized according to the classic definition of friction between two flat surfaces. Because of the complex nature of fiber friction, the frictional coefficient value strongly depends on testing conditions [7].